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מספר: Number	131887
תאריך: Date	14-09-1999
הוקדם/נדחה Ante/Post-dated	

בקשה לפטנט
Application for Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
(Name and address of applicant, and in case of body corporate-place of incorporation)

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Owner, by virtue of of an invention the title of which is

(בעברית) (Hebrew) פרו-תרופות פוספוליפידיות של תרופות אנטי-פרוליפרטיביות

(באנגלית) (English) PHOSPHOLIPID PRODRUGS OF ANTI-PROLIFERATIVE DRUGS

מבקש בזאת כי ינתן לי עליה פטנט hereby apply for a patent to be granted to me in respect thereof.

*בקשת חלוקה - Application of Division	*בקשת פטנט מוסף - Application for Patent Addition	*דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
מס' No.....	מס' No.....			
מיום dated.....	מיום dated.....			
* יפוי כח: כללי / מיוחד - רצוף בזה / עוד יוגש P.O.A.: general / individual-attached / to be filed later -				
הוגש בעניין filed in case.....				

המען למסירת מסמכים בישראל Address for Service in Israel

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Signature of Applicant

For the Applicants,

Cynthia Webb

Dr.Cynthia Webb
Patent Attorney

היום ..... בחודש ..... שנת .....
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פרו-תרופות פוספוליפידיות של תרופות אנטי-פרוליפרטיביות

PHOSPHOLIPID PRODRUGS OF ANTI-PROLIFERATIVE DRUGS

D-PHARM, LTD.

די-פארם בע"מ

PHOSPHOLIPID PRODRUGS OF ANTI-PROLIFERATIVE DRUGS

FIELD OF THE INVENTION

The present invention relates to prodrugs of anti-proliferative agents comprising a phospholipid moiety covalently linked, via a bridging group, to an anti-proliferative agent, such that the active species is preferentially released, preferably by enzymatic cleavage, at the required site of action. The invention further relates to pharmaceutical compositions comprising said prodrugs and to the use thereof for the treatment of diseases and disorders related to inflammatory and degenerative or atrophic conditions and to uncontrolled cell growth.

BACKGROUND OF THE INVENTION

Numerous compounds associated with diverse classes of chemicals are in use in cancer therapy. Common features of all these agents are cytotoxic properties that enable them to exert their anti-proliferative activity. However, the utility of anti-proliferative drugs is severely hampered by their excessive toxicity and deleterious effects on normal healthy cells of the organism.

Many anti-proliferative drugs, also known as anti-metabolites, are also very useful as anti-inflammatory agents. These drugs suppress local or even systemic inflammatory responses mediated by the immune system.

Methotrexate (MTX) is an effective anti-neoplastic drug commonly used in cancer therapy. MTX is also known as a leading anti-inflammatory drug, and is currently the main drug used in the treatment of rheumatoid arthritis (RA). It is the drug of first choice for treating children with recalcitrant juvenile RA.

Methotrexate is an analogue of dihydrofolate that inhibits dihydrofolate reductase (DHFR) thus depleting intracellular tetrahydrofolate (FH_4). FH_4 is a cofactor essential for the methylation of uracil to form thymidylate, and is thus essential for DNA synthesis and for the *de novo* synthesis of purines. MTX also

inhibits production of methionine and increases adenosine release, a potent anti-inflammatory substance, which activates adenosine A_2 receptors on inflammatory cells. MTX also profoundly inhibits TNF levels in the joints of adjuvant arthritic rats and reduces intra-articular production of PGE_2, thromboxane B2 and IL-1 in the early phase of antigen induced arthritis in rabbits. All these actions of MTX inhibit cellular proliferation, decrease formation of antibodies, and decrease formation of mediators of inflammation such as interleukins and eicosanoids. MTX therapy in RA patients also induces indirect effects such as changes in the levels of cytokines in the synovial fluid and decrease of collagenase expression. The molecular mechanism of the actions of methotrexate remains, however, largely unknown.

Another anti-metabolite widely used in cancer chemotherapy is the pyrimidine analog fluorodeoxyuridine (FUdR). FUdR is converted *in vivo* into fluorodeoxyuridylate (F-dUMP) which is an analog of the normal substrate dUMP and irreversibly inhibits thymidylate synthase.

MTX and FUdR are valuable drugs in the treatment of many rapidly growing tumors, however, their use is limited by the frequency and severity of side effects. Unwanted side effects include toxicity to all rapidly dividing normal cells, such as stem cells in the bone marrow, epithelial cells of the intestinal tract, etc. Folic acid antagonists are also toxic to developing embryos. Treatment with MTX is especially problematic in patients having chronic debilitating inflammatory diseases that require prolonged therapy, such diseases as rheumatoid arthritis, asthma, dermatological diseases such as psoriasis and gastrointestinal inflammation such as Crohn's disease. These patients may suffer from induced nephrotoxicity, due to precipitation of the drug or its metabolites in the renal tubes, and from hepatic fibrosis and cirrhosis.
In cancer therapy high doses of methotrexate may be administered with 'rescue' of host from toxicity by administration of reduced folate, leucovorin.

It would, therefore, be most advantageous to have drug derivatives that are targeted or selectively active in the diseased cells rather than in the healthy cells, thus reducing undesirable side effects. It would also be desirable to

improve the efficacy of anti-proliferative drugs and to be able to extend their usefulness to conditions that do not respond to lower doses of the drugs.

The use of prodrugs to impart desired characteristics such as increased bioavailability or increased site-specificity for known drugs is a recognized concept in the state of the art of pharmaceutical development. The use of various lipids in the preparation of particular types of prodrugs is also known in the background art. In none of those instances are the prodrugs able to achieve preferential accumulation of an anti-proliferative drug within the diseased cells of the organ, by activation with intracellular lipases. Rather, the prodrugs enable the drug to be transported to a specific site, or to be released within a specific organ, as in the case of the phospholipid prodrugs of salicylates and non-steroidal anti-inflammatory drugs disclosed in International Patent Application WO 91/16920 which, taken orally, protect the gastric mucosa and release the active principle in the gut.

In other examples of phospholipid prodrugs, the formulation of the prodrugs into liposomes or other micellar structures is the feature that enables their preferential uptake, for instance by liver cells or by macrophages as in the case of the phospholipid conjugates of antiviral drugs disclosed in International patent applications WO 93/00910 and WO 90/00555.

U.S Patent No. 5,411,947 discloses a method of converting a drug to an orally available form by covalently bonding a lipid to the drug. It discloses a lipid prodrug of 5-fluorouridine, but differs from the present invention in that the drug is attached to the lipid at the R^3 position.

U.S Patent No. 4,772,594 discloses prodrugs containing 5-Fluorouracil, but differs from the present invention in that the drug is attached to cholesterol.

U.S. Patent Nos. 5,149,794 and 5,543,389 disclose covalent polar lipid-drug conjugates to facilitate the entry of drugs into cells at pharmokinetically useful levels. In contrast to the present invention, the disclosed prodrugs are directed to certain intracellular structures and organelles due to the existence of the polar lipid carrier, and drug release from the lipid conjugate is not a requirement for the drug targeting. Moreover, it is explicitly stated that the conjugates may be

pharmacologically active themselves. Though phospholipids are included in a list of potentially useful polar lipids, the drug-phospholipid conjugates were clearly never reduced to practice and have not been suggested to be inactive derivatives of the drug.

Non-lipid analogues of methotrexate have been previously disclosed (for example Antonjuk, D. *et al., 1984* J. Chem. Soc. Perkin Trans. *1 1989*), however the derivatives are monoamides and not phospholipids as in the present invention.

International Application WO 94/22483 describes prodrugs which selectively release pharmacologically active compounds in hyperactivated cells, but does not disclose methotrexate or 5-fluorodeoxyuridine derivatives.

SUMMARY OF THE INVENTION

The object of the present invention is to provide prodrugs of anti-proliferative agents with decreased toxicity. It is a further object of the present invention to provide prodrugs of anti-proliferative agents that undergo preferential activation within the disease-affected cells and tissues.

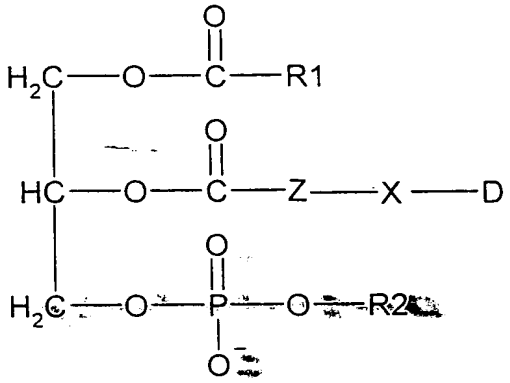
The prodrugs in accordance with the present invention comprise a phospholipid moiety covalently linked, via a bridging group, to an anti-proliferative drug residue, such that the active species is preferentially released by enzymatic cleavage at the required site of action.

According to the principles of the present invention, the anti-proliferative drug is chemically modified to produce a pharmacologically inactive prodrug, sensitive to cleavage by phospholipases produced in excess at the disease site. Hence, activation of the drug occurs at the site of the disease, whereas in healthy tissue the prodrug remains inert. The process of using an inactive prodrug that is susceptible to cleavage by the hyperactive enzymes of the pathological target tissue is referred to herein as Regulated Activation of Prodrugs (RAP).

RAP prodrugs are composed of the active drug moiety attached via a chemical linkage to a hydrophobic molecule that facilitates efficient cell

penetration. The specificity of the activation is afforded by the linkage group that is designed to be sensitive to cleavage by lipases, preferably phospholipases that are specifically elevated at the disease site. This specific cleavage of the prodrug at the disease site, results in increased efficacy at relatively lower doses, and hence, reduced side effects and toxicity. There is consequently, a higher therapeutic index (LD_{50}/ED_{50}) for the drug.

In a first aspect, the present invention provides a prodrug of the general formula I



Formula I

or a pharmaceutically acceptable salt thereof, wherein:

R1 is a saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms;

R2 is H or a phospholipid head group;

Z is saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 15 carbon atoms, which may include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms;

X is a covalent bond or selected from the group consisting of O, S, NH and C(O) groups; and

D is a residue of an anti-proliferative drug,

wherein the bound anti-proliferative drug residue is an inactive form of the drug which is selectively activated in cells and tissues with elevated phospholipase activity.

According to a currently preferred embodiment, R1 of the prodrug of the general formula I is a hydrocarbon chain having from 5 to 20 carbon atoms, more preferably 15 or 17 carbon atoms.

In another preferred embodiment R2 of the prodrug of the general formula I is selected from the group consisting of choline, ethanolamine, inositol and serine.

Preferred anti-proliferative drugs used in the prodrug of the general formula I are methotrexate and 2'-deoxy-5-fluorouridine.

Currently most preferred prodrugs according to the invention are selected from the group consisting of:

1-Stearoyl-2-[3-(α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[3-(γ-dodecylate-α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[4-(α-MTX amido)-Butanoyl]-sn-Glycero-3-Phosphatidylcholine,

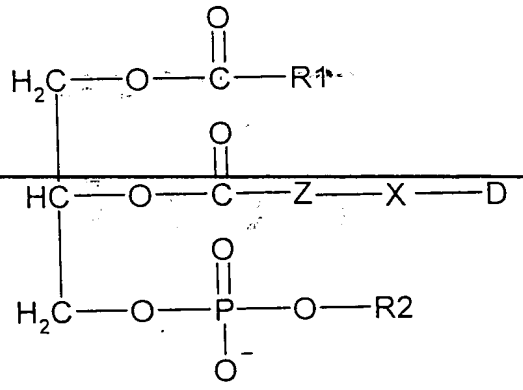
1-Stearoyl-2-[6-(α-MTX amido)-Hexanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[8-(α-MTX amido)-Octanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[8-(γ-dodecylate-α-MTX amido)-Octanoyl]-sn-Glycero-3-Phosphatidylcholine, and

1-Stearoyl-2-[(5''-2'-deoxyuridine)-3'',3''=dimethyl]-glutaroyl-sn-Glycero-3-Phosphatidylcholine.

In another aspect of the invention, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a prodrug of the general formula I



Formula I

or a pharmaceutically acceptable salt thereof, wherein:

R1 is a saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms;

R2 is H or a phospholipid head group;

Z is saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 15 carbon atoms, which may include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms;

X is a covalent bond or selected from the group consisting of O, S, NH and C(O) groups; and

D is a residue of an anti-proliferative drug,

wherein the bound anti-proliferative drug residue is an inactive form of the drug which is selectively activated in cells and tissues with elevated phospholipase activity.

The pharmaceutical composition, in accordance with the invention, may further include one or more additional anti-neoplastic agents.

In a further aspect, the present invention provides the use of a prodrug of the aforementioned formula I, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition.

In still a further aspect, the invention provides methods for treatment of diseases or disorders related to an inflammatory condition such as granulomatous disease, arthritis, rheumatoid arthritis, systemic sclerosis, asthma, psoriasis, systemic lupus erythematosus, inflammatory bowel syndrome and migraines. Also provided are methods for treatment of diseases and disorders related to a degenerative or atrophic condition, in particular central or peripheral neurological disease or disorder selected from, but not limited to, the group consisting of autoimmune, cerebrovascular and neurodegenerative diseases and disorders such as idiopathic dementia, vascular dementia, multi-infarct dementia, traumatic dementia, Alzheimer's disease, Pick's disease, Huntington's disease, dementia paralitica, Parkinson's disease, diabetic neuropathy, multiple sclerosis, amyotrophic lateral sclerosis, acute ischemia of the optic nerve, age-related macular degeneration, stroke and trauma.

Also provided are methods for treatment of a disease or disorder related to uncontrolled cell growth. Said disease or disorder may be selected from, but is not limited to, psoriasis, lymphocytic leukemia, acute myelogenous leukemia, meningeal leukemia, non-Hodgkin's lymphomas, mycosis fungoides, osteosarcoma, hydatidiform mole, trophoblastic diseases such as chorioadenoma destruens and choriocarcinoma, epidermoid cancers of the head and neck, and carcinomas of the breast, liver, lung, colon, ovary, cervix, urinary, bladder, prostate, pancreas, the gastrointestinal tract and the oropharyngeal areas. Said aforementioned methods comprise administering to a patient in need thereof a pharmaceutical composition containing a therapeutically effective amount of a prodrug of the general formula I in accordance with the invention.

DETAILED DESCRIPTION OF THE INVENTION

The prodrugs according to the invention comprise anti-proliferative agents covalently conjugated, via a bridging group, to position sn-2 of a phospholipid. The conjugated anti-proliferative drug residue is pharmacologically inactive, and regulated release of the active drug occurs at the site of a diseased tissue.

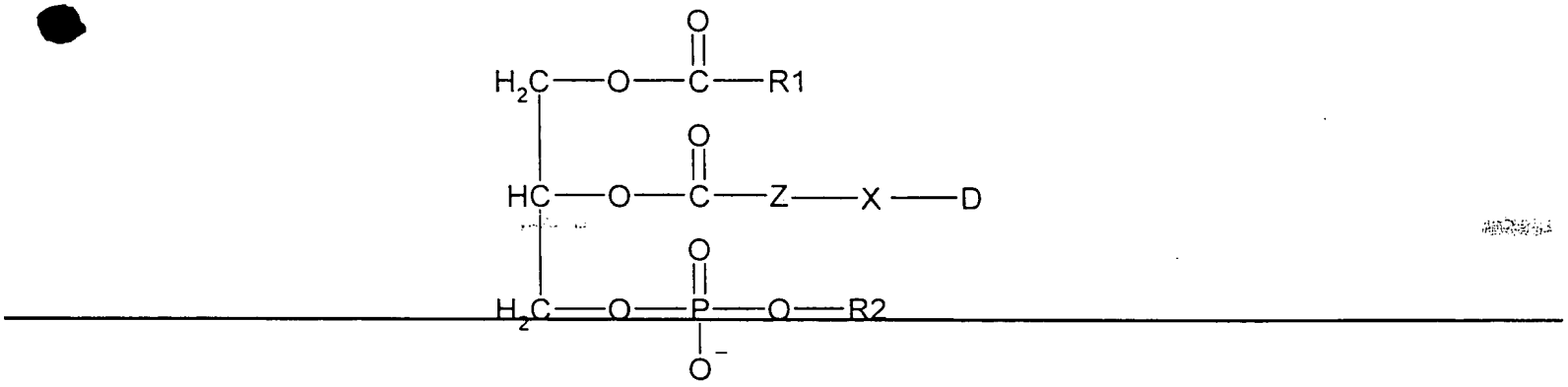
The compounds, being hydrophobic in nature, may penetrate biological membranes and barriers, thus effectively transporting the prodrug into cells or organs. The specificity of the activation of the anti-proliferative prodrug is afforded by the bridging group that is designed to be sensitive to cleavage by phospholipases (e.g. PLA_2) that are specifically elevated at the disease site. Hence, accumulation of the active drug occurs at the site of the disease, whereas, in healthy tissue there will be only a basal level of prodrug cleavage.

It should be appreciated that the novel prodrugs of the invention have a higher therapeutic index comparing to their corresponding parent drugs. Thus, while serving as effective anti-proliferative agents, the compounds of the invention exhibit reduced side effects and toxicity.

Accordingly, it is possible to extend the usefulness of these anti-proliferative agents to conditions that do not respond to lower doses of the drug and to reduce undesirable side effects by the regulated release of the active drug at the target site.

The prodrug molecules of the invention are designed in accordance with the concept of the aforementioned Regulated Activation of Prodrugs (RAP) technology, namely the linkage of the active principle in the prodrug molecule is such that renders the drug residue inactive. At the same time, the prodrug is so designed to enable the preferential release of the active anti-proliferative drug at the disease site.

Thus, in accordance with one aspect of the invention, there are provided prodrugs of the general formula I



Formula I

or a pharmaceutically acceptable salt thereof, wherein:

R1 is a saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms;

R2 is H or a phospholipid head group;

Z is saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 15 carbon atoms, which may include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms;

X is a covalent bond or selected from the group consisting of O, S, NH and C(O) groups; and

D is a residue of an anti-proliferative drug,

wherein the bound anti-proliferative drug residue is an inactive form of the drug which is selectively activated in cells and tissues with elevated phospholipase activity.

In designing a prodrug compound in accordance with the invention, the specific nature of the pathological condition to be treated should be considered. This involves determining the desired pharmacological activity to be achieved, hence the choice of the anti-proliferative drug residue D. In addition, the

phospholipid moiety may be modified in order to optimize lipophilicity of the prodrug and drug release.

The desired lipophilicity of the molecule depends on the particular site where the pharmaceutical activity is needed. Accordingly, the number of carbon atoms in the R1 hydrocarbon chain of a prodrug of the general formula I, is determined. The lipophilicity of the molecule is directly correlated to the selected hydrocarbon chain length. R1 chains according to the invention may contain 2 to 30 carbon atoms. Molecules with R1 having from 5 to 20 carbon atoms are most desirable as endowing the molecule with suitable hydrophobicity for crossing biological membranes and at the same time providing adequate substrate for the action of the phospholipase.

R1 may be a straight-chained or branched, saturated or unsaturated hydrocarbon chain, containing one or more double and/or triple bonds. One or more hydrogen atoms on the chain may be substituted, for example, by halogen atoms or by a small alkyl group such as methyl residues, with the proviso that the substituents still allow free access for the desired cleaving enzymes.

In preferred embodiments of the invention R1 is an alkyl residue of an odd number of carbon atoms. More preferably R1 is an alkyl residue of 15 or 17 carbon atoms yielding, respectively, the naturally occurring palmitoyl (C_{16}) or stearoyl (C_{18}) residues at the sn-1 position of the phospholipid.

The lipophilicity of the lipid molecule is also affected by the nature of the phospholipid head group, denoted as R2 in the prodrug of the general formula I. The phospholipid moiety may be selected from, but is not limited to, the group consisting of phosphatidic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine.

The anti-proliferative drug residue incorporated into the phospholipid prodrug may be any anti-proliferative agent that inhibits, directly or indirectly, cell growth. The drug is covalently linked, via a bridging group, to position sn-2 of the phospholipid, such that the anti-proliferative activity of the bound drug residue is significantly reduced.

In one particular embodiment, prodrugs of the invention are phospholipid derivatives of methotrexate. In a preferred embodiment, binding of the drug to the lipid-bridge moiety is specifically directed to the α– rather than γ– carboxylic group of methotrexate. It was found that binding of methotrexate via its alpha carboxylic group (herein denoted α -MTX) yields a pharmaceutically inactive methotrexate, while blocking the gamma carboxyl of methotrexate, does not greatly affect the anti-proliferative activity of the drug. Accordingly, prodrugs wherein the methotrexate or pharmaceutically acceptable derivative thereof is attached through the α carboxylic group of methotrexate, are the most preferred compounds according to the present invention. However, encompassed within the scope of the invention, are also compounds wherein the methotrexate or pharmaceutically acceptable derivative thereof is attached through the γ carboxylic group of methotrexate. In this case, in order to render the prodrug inactive, the hydrogen atom of the α carboxylic group of methotrexate is substituted by a saturated or unsaturated, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms.

Preferably, the appropriate drugs are linked to the bridging group through a carboxyl, oxy, amine or mercapto group, thus generating an ester, amido or a thio bond.

Suitable anti-proliferative drugs may include, but are not limited to, anti-metabolites such as paclitaxel, estramustine, melphalan, carmustine, nimustine, daunorubicin, doxorubicin, denopterin, methotrexate, trimetrexate, fluorouracil, fluorodeoxyuridine, 6-azauridine, flutamide, nilutamide, goserelin, leuprolide and anthralin.

In preferred embodiments of the invention the anti-proliferative drug is selected from methotrexate and 2'-deoxy-5-fluorouridine and pharmaceutically acceptable derivatives thereof.

The anti-proliferative drug residue, D, is covalently linked to the phospholipid moiety via the bridging group –C(O)-Z-X. The choice of the preferred bridging group is dependent on several considerations; it should participate in a stable covalent bond with the D moiety while lending itself to

cleavage at the target site. A preferred bridging group, is such that is resistant to cleavage under normal physiological conditions encountered by the administered compound on its way to the target site. The bridging group should not confer a steric hindrance on the enzymatic cleavage of the ester bond at position sn-2 of the phospholipid of the general formula I.

Depending on the treated phatological condition and the particular diseased cell or organ, it will be desirable at times to choose such a bridging group that will regulate the release of the active drug by facilitating or delaying its cleavage from the prodrug molecule.

Component Z of the bridging group may be a saturated or unsaturated, straight-chain or branched hydrocarbon chain having from 2 to 15 carbon atoms, which may include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms. Component X may be a covalent bond or is selected from the group consisting of O, S, NH and C(O) groups. According to a preferred embodiment, the total number of carbon atoms in the bridging group -C(O)-Z-X is at least 6 but at most 15. It was found that this length of carbon chain provides a spacer which enables good access to an enzyme, preferably phospholipase and in particular PLA_2, for digesting the ester bond at position sn-2 of the phospholipid of the general formula I. Shorter spacers, in particular bridging groups comprising less than three carbon atoms, may be problematic, by creating an unfavorable steric environment for the action of the phospholipase. A situation of steric interference may also be generated by long spacers, i.e. when the number of carbon atoms in the bridging chain is greater than 15.

It is important to note that the functional group of D through which the drug residue is bound to the bridging group may be selected from amino, hydroxyl, thiol and carboxyl groups, with the proviso that when the functional group of D is –C(O)OH, X is not a carbonyl, and when the functional group of D is –OH, $–NH_2$, –NH or -SH, X is a carbonyl group. The above restrictions are applied since some combinations of X with particular drug residues may be unfavorable as yielding a very labile bond which is spontaneously cleaved,

therefore greatly lowering the efficacy of the prodrug. Such an unfavorable combination is, for example, where the covalent bond formed is –(CO)-O-(CO)- . This is a labile bond that tends to dissociate, hence greatly reducing drug bioavailability and therapeutic effects at the target site.

The therapeutic efficacy of any particular compound according to the invention should be evaluated by a person skilled in the art considering the general knowledge in chemotherapy and the teachings of the present invention. The choice of a specific compound to be used as a prodrug according to the invention will also depend on the particular disease or disorder to be treated.

It is suggested that the release of the active drug at the target site is initiated by a first cleavage of the prodrug at position sn-2 of the phospholipid, preferably by a phospholipase, more preferably phospholipase A_2. PLA_2 was chosen for the following compelling reasons; (i) its enhanced activity is a common feature in many inflammatory processes, (ii) it is abnormally elevated during the progression of the inflammatory disease, (iii) elevated PLA_2 activity is associated with some cancers (e.g. breast and colon cancer) and (iv) under ischemic conditions, such as at the core of some solid tumors, there is an additional PLA_2 activation.

Thus, in accordance with the RAP technology, the increased PLA_2 activity in certain malignant cells and tissues and under inflammatory or degenerative conditions, leads to preferential cleavage of the prodrug and release of the drug within the diseased affected cells and tissues.

The conjugate prodrugs, in accordance with the invention, are designed to have distinct advantages over their coresponding parent drugs, including improved pharmacokinetic properties and reduced toxicity. These advantageous characteristics are manifested in higher therapeutic indices of the compounds of the invention. It is expected that, due to the aforementioned advantages, the prodrug compounds of the invention will be efficient alternative novel drugs for treating cancer and inflammatory-related diseases and disorders.

The first cleavage at position sn-2 of the phospholipid, may further facilitate the following cleavage necessary for releasing the active moiety of the anti-proliferative drug from the bridging group. This second cleavage may be enzymatically or non-enzymatically executed. Candidate enzymes for performing the second cleavage may include an amide hydrolase, esterase or any other suitable enzyme functionally available at the diseased site. Alternatively, the release of an active anti-proliferative drug from the prodrug compound may be initiated by any cleavage at position sn-2 of the phospholipid that eventually leads to release of an active drug. Moreover, under certain circumstances the active drug released may be different from the original parent drug molecule. This includes drug derivatives wherein a chemical group(s) has been removed from or added to the original anti-proliferative residue D. These cases are also included within the concept of the invention provided that the resulted drug derivative preserves therapeutic activity. Preferably the cleavage process of the molecule of the invention is initiated specifically at the diseased cells, thus generating a highly specific and highly effective drug released at the desired target site.

Irrespective of the exact mechanism of action, it is evident that the novel prodrugs of the invention have an enhanced therapeutic profile. Furthermore, they are more effective than their corresponding parent drugs in at least two aspects: (i) increased specificity, and (ii) decreased side effects. The prodrugs of the invention may enable extending the usefulness of anti-proliferative agents to conditions that do not respond to lower doses of drugs as well as reducing undesirable side effects by preferential releasing of the active drug at the diseased site.

In accordance with another aspect of the invention, there are provided pharmaceutical compositions comprising as an active ingredient a prodrug of the general formula I, together with a pharmaceutically acceptable diluent or carrier as are known in the art.

In particularly preferred embodiments of the invention, there are provided pharmaceutical compositions comprising phospholipid derivatives of

methotrexate and fluorodeoxyuridine (herein respectively denoted as DP-MTX and DP-FUdR). Specific derivatives are denoted by numerical suffixes.

The linkage of the anti-proliferative drug moiety to the phospholipid is via a bond that is susceptible to cleavage by phospholipase. Thus, at diseased site characterized by elevated activity of phospholipase, DP-MTX prodrug, for example, is cleaved to release free methotrexate or a therapeutically active derivative thereof. The released drug will inhibit dihydrofolate reductase thus depleting intracellular tetrahydrofolate (FH_4). DNA synthesis and *de novo* purine synthesis will, therefore, also be shut down resulting in inhibition of highly proliferative cells, such as cancer cells. Similarly, at the site of inflammation, the specific release of methotrexate leads to inhibition of leukocyte activation and migration, hence suppressing the inflammatory condition. In the case of rheumatoid arthritis, DP-MTX displays improved intra-articular retention in the inflamed synovium and reduced clearance from the joints due to the presence of its hydrophobic phospholipid moiety.

In a similar way DP-FUdRs, the novel phospholipid derivatives of fluorodeoxyuridine, demonstrate improved properties as anti-proliferative prodrugs. The fact that these prodrugs are capable of selectively releasing their pharmaceutically active component at the target site should enable a significant reduction in therapeutic dose coupled with a reduction in the frequency of administration.

The pharmaceutical compositions of the invention are useful in the treatment of diseases and disorders related to inflammatory, degenerative or atrophic conditions and in the treatment of diseases and disorders related to uncontrolled cell growth.

In one preferred embodiment, the pharmaceutical compositions are applicable in the treatment of neoplastic growths that may be benign or malignant growths including primary tumors as well as secondary tumors such as metastases. The neoplastic growths include, but are not limited to, psoriasis, lymphocytic leukemia, acute myelogenous leukemia, meningeal leukemia, non-Hodgkin's lymphomas, mycosis fungoides, osteosarcoma, hydatidiform mole,

trophoblastic diseases such as chorioadenoma destruens and choriocarcinoma, epidermoid cancers of the head and neck, carcinomas of the breast, liver, lung, colon, ovary, cervix, urinary, bladder, prostate, pancreas and cancers of the gastrointestinal tract and the oropharyngeal areas.

In another preferred embodiment, the pharmaceutical compositions are useful for treating diseases and disorders related to an inflammatory condition including, but not limited to, granulomatous disease, arthritis, rheumatoid arthritis, systemic sclerosis, asthma, psoriasis, systemic lupus erythematosus, inflammatory bowel syndrome and migraines.

In yet another preferred embodiment, the prodrugs and pharmaceutical compositions provided by the invention are useful for treating diseases and disorders related to a degenerative or atrophic condition.

Said degenerative or atrophic condition may include, but is not limited to, a central or peripheral neurological disease or disorder including, but not limited to, autoimmune, cerebrovascular and neurodegenerative diseases and disorders such as idiopathic dementia, vascular dementia, multi-infarct dementia, traumatic dementia, Alzheimer's disease, Pick's disease, Huntington's disease, dementia paralitica, Parkinson's disease, diabetic neuropathy, multiple sclerosis, amyotrophic lateral sclerosis, ocular conditions such as acute ischemia of the optic nerve (AION) and age-related macular degeneration, and other pathological conditions such as stroke and trauma.

It should be appreciated that the prodrugs and pharmaceutical compositions in accordance with the invention may be useful in curative as well as in preventive medical treatments. For example, uncontrolled cell growth, inflammatory and degenerative processes possibly leading to pathological conditions and diseases in the nervous system and blood vessels, may be prevented or inhibited by the prodrugs of the invention. In a particular case, the phenomenon of restenosis, frequently developed following invasive procedures used in the treatment of arteriosclerosis diseases, may be ameliorated or prevented by applying a therapeutically effective amount of an anti-proliferative agent in accordance with the invention. The invasive

procedures may include, but are not limited to, vascular surgery procedures such as percutaneous transluminal coronary angioplasty (PTCA) and bypass operations.

The pharmaceutical compositions may include therapeutically effective amounts of a prodrug in accordance with the invention together with one or more additional agents known to be effective in the treatment of a particular disease or disorder. For example, beneficial effects have been observed when methotrexate is used as part of a combination therapy in patients with carcinoma or Burkitt's and other non-Hodgkin's lymphomas. Methotrexate is routinely used in combination with cyclophosphamide and fluorouracil in the treatment of breast cancer, in combination with cisplatin and/or doxorubicin in the treatment of cancer of the bladder, and in combination with cisplatin and bleomycin in the treatment of carcinomas of the cervix and head and neck. In these and other combinations it is possible to substitute one or more of the active drugs for a prodrug of the invention comprising the corresponding drug residue which may endow the medicament with an additional therapeutic value.

The pharmaceutical compositions may be in a liquid, aerosol or solid dosage form, and may be formulated into any suitable formulation including, but not limited to, solutions, suspensions, micelles, emulsions, microemulsions, aerosols, ointments, gels, suppositories, capsules, tablets, and the like, as will be required for the appropriate route of administration.

In yet another aspect, the present invention provides methods for treating pathological conditions related to uncontrolled cell growth, inflammatory and degenerative or atrophic conditions. Said methods comprise administering to an individual in need thereof a therapeutically effective amount of a prodrug of the general formula I or a pharmaceutical composition in accordance with the invention.

The term "therapeutically effective amount" used in the specification refers to the amount of a given prodrug compound according to the invention which antagonizes or inhibits, directly or indirectly, activities associated with inflammatory, degenerative or atrophic processes or uncontrolled cell growth,

hence providing either a subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

Any suitable route of administration is encompassed by the invention including, but not being limited to, oral, intravenous, intraperitoneal, intramuscular, subcutaneous, inhalation, intranasal, topical, rectal or other known routes. In preferred embodiments, the pharmaceutical compositions of the invention are orally or intravenously administered.

The dose ranges for administration of the compositions of the invention are those large enough to produce the desired anti-proliferative effect. The dosing range of the prodrug varies with the specific drug used, the treated pathological condition, the route of administration and the potency of the particular prodrug molecule in releasing the drug at the specific target site. The dosage administered will be dependent upon the age, sex, health, weight of the recipient, concurrent treatment, if any, frequency of treatment and the nature of the effect desired. Dosage regimen and means of administration will be determined by the attending physician or other person skilled in the art.

The invention will now be illustrated by the following non-limiting examples:

EXAMPLES

I. CHEMICAL EXAMPLES

Example 1: General synthesis of phospholipid α-monoamide derivatives of methotrexate (DP-MTX monoamides)

A general procedure for the synthesis of DP-MTX monoamide compounds is described below and illustrated in scheme 1. It is important to note that in this protocol a selective binding to the α and γ positions of the methotrexate has been achieved.

The synthesis pathway consists of six steps. Step 1 is protection of an amino group with a benzyloxycarbonyl resulting in the corresponding Z-amino acid. In step 2 the Z-amino acid is converted into the Z amino acid anhydride. Step 3 is synthesis of DP-amino acid, namely the formation of lipid derivative comprising the amino acid with protected amino group and a lyso-lecithin. The DP-amino acid is hydrogenated in step 4. In step 5 the mixed anhydride of methotrexate or its γ-esters are prepared by reacting methotrexate (γ-esters) with isobutyl chloroformate in the presence of triethyl amine. In the last stage, step 6, the final product is obtained by reacting the mixed anhydride of methotrexate with 1-acyl-2-(ω-amino) acyl-Sn-glycero-3-phosphatidylcholine in the presence of triethylamine as a catalyst.

For the sake of clarity, each of the intermediary steps will be described below in details.

Stage 1. Protection of the amino group of amino acid (Preparation of Z-amino acid).

To a mixture of 0.1 mol corresponding amino acid (3-aminopropanoic acid, 4-aminobutanoic acid, 6-aminohexanoic acid, 8-aminooctanoic acid etc.) in ethanol (25 ml) in round-bottom flask (250 ml) equipped with a magnetic stirrer and dropped funnel, a solution of NaOH (8.8g., 0.22 mol) in 100 ml water is added and the mixture is stirred by magnetic stirrer until fully dissolved. The obtained solution is cooled to 0^0C in an ice-water bath, and benzyl chloroformate (27.4g , 0.15 mol) is added drop wise over 30 min. The reaction mixture is stirred for 3 hours at 0^0C. Subsequently, about 100 ml water is added to the reaction solution and the mixture is poured into separated funnel. The water solution is extracted with diethyl ether (3X 50 ml). The water phase is separated and acidified with HCl (3N) to pH=1 while cooling in an ice-water bath. If a precipitate is formed, it is filtered, washed with water and dissolved in 100 ml chloroform. The chloroform solution is dried with sodium sulfate for two hours. Then the sodium sulfate is separated from the chloroform solution by filtration and the solvent is evaporated in evaporator under vacuum. The

residue is washed with hexane, and dried overnight in vacuum over phosphorus pentoxide (P_2O_5).

If a precipitate is not formed, or in order to maximize the product yield, the acidified aqueous fraction is washed with chloroform (2X 50 ml). The chloroform extracts are combined and washed with water (50ml). The following operations with this solution are the same as for the above-described chloroform solution of the precipitate, namely, drying with sodium sulfate for two hours, then separating the sodium sulfate from the chloroform solution by filtration and evaporating the solvent in evaporator under vacuum. The residue is then washed with hexane, and dried overnight in vacuum over phosphorus pentoxide.

All products were analyzed on TLC as follows:

TLC analysis. Silica gel 60 on aluminum sheet. Eluent is chloroform-methanol (4:1 v/v). Indicator is a spray of the composition: 4-methoxybenzaldehyde (10ml); absolute ethanol (200ml), 98% sulfuric acid (10ml) and glacial acetic acid (2ml). The chromatogram is sprayed with the indicator and then charred using hot air at 150-180^{0}C.

The following are specific intermediate products obtained at the end of stage 1 of the synthesis procedure:

3-[N-(Benzyloxycarbonyl)amino]propanoic acid.. C_6H_5-CH_2-O-C(O)-NH-CH_2-CH_2-COOH.

White solid. Yield 60%. TLC analysis: One spot R_f 0.7.

^{1}H NMR (CD_3OD), δ(ppm): 2.46-2.52 (t, 2H, α-CH_2 group of alanine), 3.29-3.39 (t, 2H, β-CH_2 group of alanine) 5.06 (s, 2H, benzylic CH_2 group), 7.27-7.32 (m, 5H, C_6H_5 group).

4-[N-(Benzyloxycarbonyl)amino]butanoic acid. $C_6H_5CH_2$-O-C(O)-NH-$(CH_2)_3$-COOH.

White solid. Yield 60%. TLC analysis: One spot. R_f 0.7.

^{1}H NMR (CD_3OD), δ(ppm): 1.71-1.82 (m, 2H), 2.28-2.34 (m, 2H), 3.10-3.17 (t, 2H), 5.06 (s, 2H), 7.26-7.34 (m, 5H).

5-[N-(Benzyloxycarbonyl)amino]valeric acid. $C_6H_5CH_2$-O-C(O)-NH-$(CH_2)_4$-COOH.

White solid. Yield 60%. TLC analysis: One spot. R_f=0.7.

^{1}H NMR (CD_3OD), δ(ppm): 1.45-1.50 (m, 2H), 1.56-1.62 (m, 2H), 2.25-2.31 (t, 2H), 3.08-3.13 (t, 2H), 5.05 (s, 2H), 7.26-7.34 (m, 5H).

6-[N-(Benzyloxycarbonyl)amino]hexanoic acid. $C_6H_5CH_2$-O-C(O)-NH-$(CH_2)_5$-COOH.

White solid. Yield 50%. TLC analysis: One spot. R_f 0.7.

^{1}H NMR (CD_3OD), δ(ppm): 1.30-1.63 (m, 6H), 2.24-2.30 (t, 2H), 3.07-3.13 (t, 2H), 5.05 (s, 2H), 7.29-7.34 (m, 5H).

8-[N-(Benzyloxycarbonyl)amino]octanoic acid. $C_6H_5CH_2$-O-C(O)-NH-$(CH_2)_7$-COOH.

White solid. Yield 50%. TLC analysis: One spot. R_f 0.7.

^{1}H NMR (CD_3OD), δ(ppm): 1.32 (bs, 6H), 1.47-1.50 (m, 2H), 1.53-1.59 (m, 2H), 2.23-2.29 (t, 2H), 3.06-3.12 (t, 2H), 5.05 (s, 2H), 7.29-7.34 (m, 5H).

Stage 2. Synthesis of Z-amino acid anhydride.

The solution of the corresponding Z-aminoacid produced at stage 1 (0.05 mol) in freshly distilled dichloromethane (25 ml) is introduced, under an atmosphere of argon, into double-neck round-bottom equipped with magnetic stirrer and dropped funnel. A solution of dicyclohexylcarbodiimide (DCC) (0.0325 mol) in 25 ml of freshly distilled dichloromethane, also under argon, is added drop wise, with stirring, to the solution of Z-amino acid. After 20 min of stirring, the obtained precipitate of urea is filtered and the solution evaporated under vacuum. The crude residue is washed with hexane (2X 20 ml) to remove remaining DCC and then dried in vacuum.

TLC analysis: The same procedure is used for TLC analysis of anhydride of the all Z-amino-acids. Silica gel 60 on aluminum sheet. Fluent is the mixture of chloroform with methanol (9:1 v/v). For indication, ninhydrine spray is used on the chromatogram followed by charring with hot air (100-150^{0}C).

The following are specific intermediate products obtained at the end of stage 2:

Anhydride of Z-(3-amino)propanoic acid. White solid. Yield is 70%.

TLC analysis: One spot R_f 0.8.

Anhydride of Z-(4-amino)butanoic acid. White solid. Yield is 70%.

TLC analysis: One spot. R_f 0.8.

Anhydride of Z-(5-amino) valeric acid. White solid. Yield is 70%.

TLC analysis: One spot. R_f 0.8.

Anhydride of Z-(6-amino)hexanoic acid. White solid. Yield is 70%.

TLC analysis: One spot. R_f 0.8.

Anhydride of Z-(8-amino)octanoic acid. White solid. Yield is 75%.

TLC analysis: One spot. R_f 0.85.

Stage 3. Preparation of 1-acyl-2- (Z-amino)acyl-sn-glycero-3-phosphotidylcholine.

The anhydride of the corresponding Z-amino acid, 0.01 mol dissolved in 150 ml of freshly distilled chloroform, is introduced, under an inert atmosphere of argon, into a single-neck round-bottom flask (250 ml) equipped with a magnetic stirrer. To this solution 0.01 mol (1.22 g) 4-(dimethylamino)pyridine (DMAP) in 25 ml chloroform is added, followed by addition of a suspension of 0.0056 moles lyso-lecithin in 50 ml of chloroform. The reaction mixture is vigorously stirred for 3-5 hours at room temperature. The lyso-lecithin dissolves and reaction mixture becomes transparent after about 2 hours of stirring. The reaction is monitored by TLC using silica gel 60 on aluminum sheet, the eluent is chloroform:methanol:water, 65:35:5, the indicator is a spray of the composition: 4-methoxybenzaldehyde (10 ml), absolute ethanol (200 ml), 98% sulfuric acid (10 ml) and glacial acetic acid (2 ml). The chromatogram is sprayed with the indicator followed by charring with hot air at 150^{0}C. The reaction is assumed to be complete and stopped when all the lyso-lecithin has disappeared. The reaction mixture is then transferred into a separating funnel and washed with a solution of 1% HCl (3x 50 ml), then with saturated solution of sodium bicarbonate (3x 50 ml) and finally with water (3x 50 ml). The

obtained product in the organic solution is dried over sodium sulfate and then filtered. The solvent is evaporated at 30^0C in vacuo and the residue is washed with hexane and left to dry overnight under vacuum. The resulted molecule 1-acyl-2-(Z-amino)acyl-sn-glycero-3-phosphatidylcholine is the main product of the reaction.

TLC analysis: Silica gel 60 on aluminum sheet. Eluent is chloroform/methanol/water (65:35:5 v/v). Indicator is a spray of the composition: 4-methoxybenzaldehyde (10 ml), absolute ethanol (200 ml), 98% sulfuric acid (10 ml) and glacial acetic acid (2 ml). The chromatogram is sprayed with the indicator and then charred using hot air at 100-150^{0}C.

The following are specific intermediate products obtained at the end of stage 3:

1-Stearoyl-2-{3'-[N-(Benzyloxycarbonyl)amino]}propanoyl-sn-glycero-3-phosphatidyl choline.

White wax. Yield 70%. TLC analysis: One spot. R_f 0.55

1-Stearoyl-2-{4'-[N-(Benzyloxycarbonyl)amino]}butanoyl-sn-glycero-3-phosphatidyl choline.

White wax. Yield 70%. TLC analysis: One spot. R_f 0.55.

1-Stearoyl-2-{5'-[N-(Benzyloxycarbonyl)amino]}valeroyl-sn-glycero-3-phosphatidyl choline.

White wax. Yield 65%. TLC analysis: One spot. R_f 0.55.

1-Stearoyl-2-{6'-[N-(Benzyloxycarbonyl)amino]}hexanoyl-sn-glycero-3-phosphatidyl choline.

White wax. Yield 65%. TLC analysis: One spot. R_f 0.55.

1-Stearoyl-2-{8'-[N-(Benzyloxycarbonyl)amino]}octanoyl-sn-glycero-3-phosphatidyl choline.

White wax. Yield 65%. TLC analysis: One spot. R_f 0.55.

Stage 4. Reduction of amide group of amino acyl lipid.

The obtained 1-stearoyl-2-{ω-[(N-Benzyloxycarbonyl)amino]}acyl-3-phosphatidyl-choline (0.0025 mol) is dissolved in a mixture of 100 ml methanol

and 5 ml acetic acid. The solution is introduced into round bottom double neck flask (200 ml) equipped with a magnetic stirrer, under an atmosphere of argon. Pd/C (0.5 g) is added to the solution and hydrogen is blown through the reaction mixture for 4 hours. The reaction proceeding is monitored by TLC analysis under the following conditions: silica gel 60 on aluminum sheet, eluent is the mixture of chloroform/methanol/water (65:35:5 v/v), indicator is a spray of the composition: p-methoxybenzaldehyde (10 ml), absolute ethanol (200 ml), 98% sulfuric acid (10 ml) and glacial acetic acid (2 ml). The chromatogram is sprayed with the indicator and then charred using hot air at 100-150^{0}C.

The reaction assumed to be complete and hydrogenation is stopped after the corresponding 1-stearoyl-2-Benzyloxycarbonylaminoacyl-sn-glycero-phosphatidylcholine has disappeared from the reaction mixture. The reaction mixture is then filtered through celete to remove the Pd/C, evaporated at 30^{0}C, under vacuum. The crude residue is washed with ether (3x 30 ml) and dried in vacuo overnight.

Conditions of the TLC analysis are the same as indicated above. The following are specific intermediate products obtained at the end of stage 4:

1-Stearoyl-2-(3-amino)propanoyl-sn-glycero-3-phosphatidylcholine, acetic acid.

White wax. Yield 70%. TLC analysis: One spot. R_f 0.2.

1-Stearoyl-2-(4-amino)butanoyl-sn-glycero-3-phosphatidylcholine, acetic acid.

White wax. Yield 70%. TLC analysis: One spot. R_f 0.2.

1-Stearoyl-2-(5-amino) valeroyl-sn-glycero-3-phosphatidylcholine, acetic acid.

White wax. Yield 65%. TLC analysis: One spot. R_f 0.2.

1-Stearoyl-2-(6-amino)hexanoyl-sn-glycero-3-phosphatidylcholine, acetic acid.

White wax. Yield 65%. TLC analysis: One spot. R_f 0.2.

1-Stearoyl-2-(8-amino)octanoyl-sn-glycero-3-phosphatidylcholine, acetic acid.

White wax. Yield 65%. TLC analysis: One spot. R_f 0.2.

Stage 5. Synthesis of the mixed anhydride of methotrexate.

Freshly distilled dimethylformamide (DMF) (25 ml) and THF (75 ml) is introduced, under atmosphere of argon, into a double-neck round-bottom flask (150 ml), equipped by magnetic stirrer and thermometer. Methotrexate (MTX; 0.454g., 1 mmol) and triethylamine (0.182g., 239μkl, 1.8 mmol) are added, under the same conditions, and dissolved in the solvent with stirring. The reaction mixture is then cooled to -25^{0}C in dry ice-acetone bath. Isobutyl chloroformate (0.166 g., 163 μl, 1.2 mmol) is added dropwise to the reaction mixture over 30 min. During this procedure and the following 30-min, the temperature of the reaction mixture is maintained at -25^{0}C. The obtained product is a white precipitate, which is not extracted from reaction mixture but was directly used in the next stage of the synthesis.

Stage 6. Synthesis of α-monoamide of methotrexate (DP- α-MTX monoamide)

A solution comprising the corresponding 1-stearoyl-2-ω-aminoacyl-sn-glycero-3-phosphatidylcholine of stage 4, acetic acid (0.589g., 0.9 mmol) and triethylamine (0.182g., 239 μl, 1.8 mmol) in 30 ml of dry freshly distilled chloroform is added dropwise to the reaction mixture of stage 5 for 30 min at -25^{0}C. The reaction mixture is stirred for additional one hour at -25^{0}C, and then for overnight at room temperature. The solvents are removed in evaporator under reduced pressure. The obtained residue is a thick viscous liquid. Diethyl ether (50 ml) is added to this liquid and the mixture is stirred. The product is gradually transformed into a yellow powder which is filtered and washed with diethyl ether (3x 20 ml). The crude product is purified by column chromatography as follows: 450 g. crude product is dissolved in 50 ml of methanol, followed by addition of 11.0 g. dry silica gel. The mixture is swirled, and then the volatile liquid is evaporated under reduced pressure to yield free-flowing yellow finely divided solid. The obtained solid is packed on top of a silica column (3x30 cm) (100g. silica gel per 1g of crude product). The product is eluted in succession with the solution MeOH:H_2O of variable composition:

first fraction is 100:0 (v/v) (1L), second fraction is 99:1 (v/v) (1L), third fraction is 98:2 (v/v) (1L), and fourth fraction is 98:3 (v/v) (2L). The fractions, which contain the product (the determination is carried out by TLC analysis), are combined and the solvent is evaporated under reduced pressure. The obtained product (about 250 mg) is dissolved in mixture of methanol (10 ml) and chloroform (250 ml). The solution is washed with 1% HCl (3x 20 ml) and then with water (3x 20 ml). To achieve better separation of the aqueous and organic phases, isopropanol (about 25% of the volume of solution) is added. Iso-propanol addition also promotes transition of the product into the organic phase. The organic layers are combined and dried over sodium sulfate. The sodium sulfate is filtered off and the solvent is distilled under reduced pressure. The obtained product is dried under vacuum for 3 hours.

Examples of resulted final products and their analyses are:

1-Stearoyl-2-[3-(α-MTX amido)]-proponoyl-sn-glycero-3-phosphatidylcholine. $C_{49}H_{79}N_{10}O_{12}P$.

Yield 35%. Yellow solid. Decompose at 200^{0}C without melting. pH 5.1.

TLC analysis: Silica gel on aluminum plates. Eluent is CH_3OH:H_2O (98:2, v/v). Indication by UV-Vis spectrum. One spot, R_f 0.16.

MS(FAB). $C_{49}H_{79}N_{10}O_{12}P$. Main peak (+FAB) is 1031.2.

^{1}H NMR (CD_3OD), δ (ppm): 0.89 (t, 3H), 1.22-1.29 (broad s, 30H), 1.53 (m, 2H), 2.08 (m, 2H), 2.35 (m, 2H), 2.60 (t, 2H), 2.50 (t, 2H), 3.21 (s, 3H), 3.38 (s, 9H), 3.47 (t, 2H), 3.93-4.31 (m, 8H), 4.41 (m, 1H), 4.80 (s, 2H), 5.21 (m, 1H), 6.84 (d, 2H), 7.76 (d, 2H), 8.60 (s, 1H).

^{31}P NMR (CD_3OD), δ (ppm): -3.3 (s).

Chemical analysis: $C_{49}H_{79}N_{10}O_{12}P.HCl.3H_2O$. Calculated: C 52.47%, H 7.55%, N 12.49%, P 2.76%, Cl 3.16%. Found: C 52.92%, H 7.76%, N 12.21%, P 2.46%, Cl 3.08%.

1-Stearoyl-2-[6-(α-MTX amido)]-hexanoyl-sn-glycero-3-phosphatidylcholine

$C_{52}H_{85}N_{10}O_{12}P$.

Yield 40%. Yellow solid. Decomposes without melting at 200^{0}C. pH 5.0.

TLC analysis: Silica gel on aluminum plates. Eluent is MeOH:H_2O (98:2, v/v). Indication is UV-Vis spectra. One spot R_f 0.18.

MS (FAB): $C_{52}H_{85}N_{10}O_{12}P$ 1073.3 (+FAB) is main peak.

^{1}H NMR (CD_3OD), δ (ppm): 0.86-0.89 (t, 3H), 1.22-1.30 (broad s, 32H), 1.51-1.55 (m, 4H), 2.06-2.10 (m, 4H), 2.33-2.36(m, 2H), 2.58-2.62 (t, 2H), 2.49-2.52 (t, 2H), 3.22 (s, 3H), 3.38 (s, 9H), 3.45-3.48 (t, 2H), 3.92-4.35 (several m, 8H), 4.40-4.42 (m, 1H), 4.79 (s, 2H), 5.20 (m, 1H), 6.82-6.84 (d, 2H), 7.74-7.76 (d, 2H0, 8.58 (s, 1H).

^{31}P NMR (CD_3OD), δ (ppm): -3.7 (s).

Chemical analysis: $C_{52}H_{85}N_{10}O_{12}P.2HCl.5H_2O$: Calculated: C 50.48%, H 7.84%, N 11.33%, P 2.51%, Cl 5.74%. Found: C 50.45%, H 7.22%, N 11.46%, P 2.43%, Cl 5.43%.

Scheme 1. Preparation of phospholipid-α-monoamide derivatives of methotrexate.

in scheme 1:

R - denotes a hydrocarbon chain having from 2 to 30 carbon atoms.

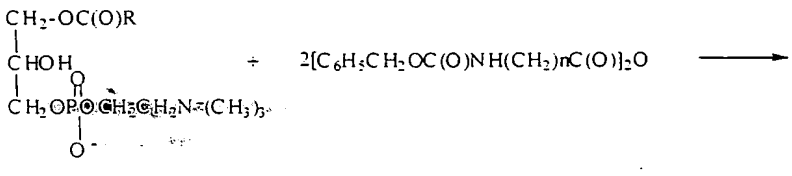
R^1 - is H, CH_3 or $C_{12}H_{25}$.

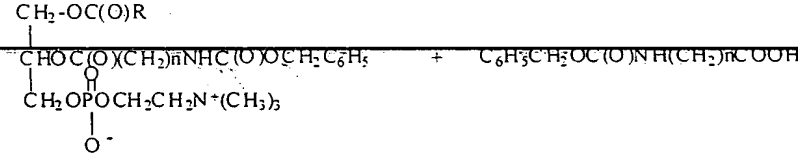
n - is an integer from 2 to 15.

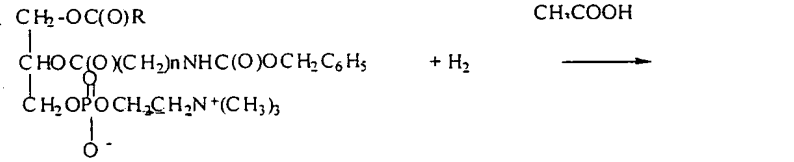
1. $C_6H_5CH_2OC(O)Cl + H_2N(CH_2)nCOOH \longrightarrow C_6H_5CH_2OC(O)NH(CH_2)nCOOH + HCl$

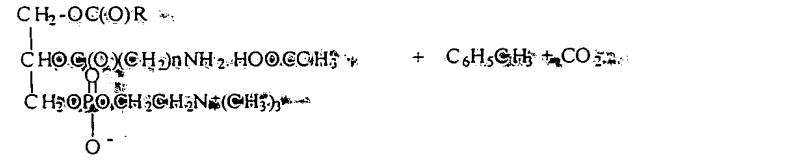
2. $2\ C_6H_5CH_2OC(O)NH(CH_2)nCOOH \quad +c\text{-}C_6H_{11}N=C=NC_6H_{11}\text{-}c \longrightarrow$

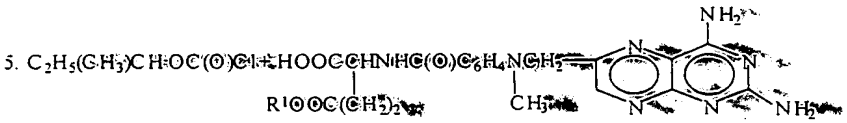
$[C_6H_5CH_2OC(O)NH(CH_2)nC(O)]_2O + (c\text{-}C_6H_{11}NH)_2CO$

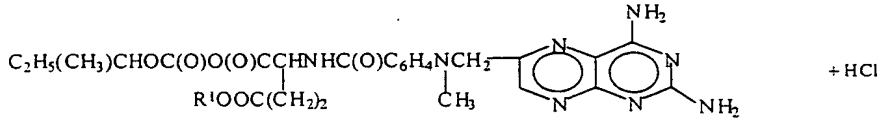
3. 

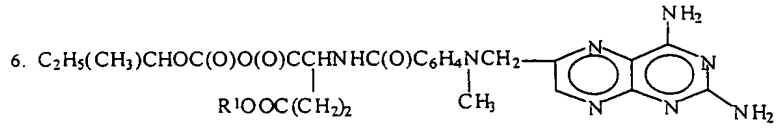


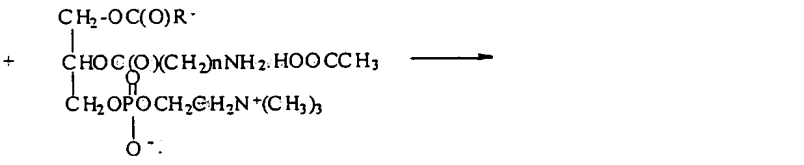
4. 

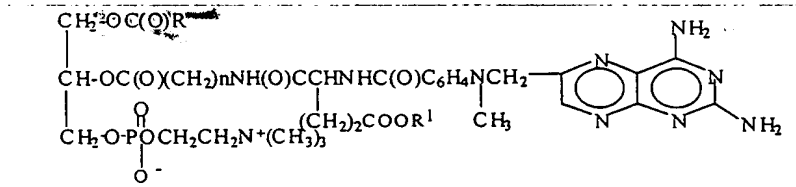


5. 



6. 





+ $C_2H_5(CH_3)CHOH + CO_2$

Example 2: General synthesis of phospholipid ester derivatives of methotrexate (DP-MTX esters)

The synthetic procedure for the preparation of DP-MTX-esters is a three-stage process.

Stage 1. Synthesis of ω-bromoalkylcarboxylic anhydride.

The solution of the corresponding ω-bromoalkyl acid (0.05 mol) in freshly distilled dichloromethane (25 ml) is introduced, under an inert atmosphere of argon, into double-neck round-bottom equipped with magnetic stirrer and dropping funnel. A solution of dicyclohexylcarbodiimide (DCC) (0.0325 mol) in 25 ml freshly distilled dichloromethane, also under argon, is added drop wise, with stirring, to the solution of Z-amino acid. After 20 min of stirring, the produced urea precipitate is filtered and the obtained solution is evaporated under vacuum. The crude residue is washed with hexane (2x 20 ml) to remove remaining DCC and then dried in vacuum.

Stage 2. Preparation of 1-acyl-2-ω-bromoalkylcarboxy-sn-glycero-3-phosphatidylcholine.

0.01 mol of the corresponding ω-bromoalkyl-carboxylic anhydride (obtained in stage 1) dissolved in 150 ml freshly distilled chloroform, is introduced, under an inert atmosphere of argon, into a single-neck round-bottom flask (250 ml) equipped with a magnetic stirrer. To this solution 0.01 mol (1.22 g) 4-(dimethylamino)pyridine (DMAP) in 25 ml chloroform is added, followed by addition of a suspension of 0.0056 moles lyso-lecithin in 50 ml chloroform. The reaction mixture is vigorously stirred for 3-5 hours at room temperature. The lyso-lecithin dissolves and reaction mixture becomes transparent after about 2 hours of stirring. The reaction is monitored by TLC using silica gel 60 on aluminum sheet, the eluent is chloroform:methanol:water, 65:35:5, the indicator is a spray of the composition: 4-methoxybenzaldehyde (10 ml), absolute ethanol (200 ml), 98% sulfuric acid (10 ml) and glacial acetic acid (2 ml). The chromatogram is sprayed with the indicator followed by charring with hot air at 150^0C. The reaction is assumed to be complete and is stopped when all the lyso-lecithin has disappeared. The reaction mixture is then

transferred into a separating funnel and washed with a solution of 1% HCl (3x 50 ml), then with saturated solution of sodium bicarbonate (3x 50 ml) and finally with water (3x 50 ml). The organic phase is dried over sodium sulfate and then filtered. The solvent is evaporated at 30^0C in vacuo and the obtained residue is washed with hexane and left to dry overnight under vacuum. The desired molecule 1-acyl-2-ω-bromoalkylcarboxy-sn-glycero-3-phosphatidylcholine is the main product of the reaction.

Stage 3. Synthesis of α-monoesters of methotrexate (DP-α-MTX esters)

A solution of 1-Stearoyl-2-ω-bromoalkylcarboxy-sn-glycero-3-Phosphocholine (0.016 mole) in 50 ml Dimethyl sulfoxide (DMSO) (freshly distilled over CaH_2) is added to a mixture of methotrexate free acid (0.008 mole) and $CsCO_3$ (0.008 mole) in DMSO (150 ml) under an atmosphere of argon. The reaction mixture is stirred at room temperature for 48 hours. The solvent is evaporated under vacuum (1-2 mm Hg, 50^0C). The obtained residue is acidified with 1% HCl to pH=3, followed by an overnight incubation at 0^0C. The mixture is then filtered, washed with water and dried over P_2O_5 under vacuum. The resultant product is 1-stearoyl-2-[ω-(α-MTX)alkyl]-oxycarbonyl-sn-glycero-3-phosphocholine. This crude product is purified by column chromatography (1 g. crude product per 100 g. Silica gel 60) using the eluent: $CHCl_3$: MeOH : H_2O (v/v 40:9:1)

Example 3: Synthesis of phospholipid derivatives of 2'-deoxy-5-fluorouridine (DP-5FUdR)

The procedure for the synthesis of DP-5FUdR compounds of the invention is exemplified below by the synthesis of the specific compound 1-stearoyl-2-[(2'-deoxy-5''-fluorouridinyl)-3'',3''-dimethyl]glutaroyl-sn-glycero-3-phosphocholine. The detailed description of the synthesis is described below and illustrated in scheme 2. The products of the different stages of the synthesis are denoted as compounds **(1)** to **(5)**.

Stage 1. Preparation of 5′-O-Trityl 5-fluoro-2′-deoxyuridine (1).

5-Fluoro-2′-deoxyuridine (2 g, 8.8 mmol) was dissolved in 40 ml dry pyridine under an atmosphere of nitrogen. Trityl chloride (3.6 g, 12.6 mmol) was added and the reaction mixture stirred at room temperature for 48 hours. The reaction was quenched with methanol (20 ml), and then the mixture was concentrated to dryness under reduced pressure. The compound was purified on silica gel (flash), using chloroform/methanol (97/3) as an eluent. 4.1 g of the product **(1)** was obtained as a colorless, crystalline solid. Yield – 99%.

Stage 2. Preparation of 3′-O-Levulinoyl 5-fluoro-2′-deoxyuridine (2).

The mixture of levulinic acid (2.32 g, 20 mmol),N,N′-dicyclohexylcarbodiimid (2.06 g, 10 mmol) and ether (60 ml) was stirred at room temperature for 5 hours, followed by filtration and evaporation of the solvent. The obtained compound was dissolved in anhydrous pyridine (10 ml). To the solution, compound **(1)** (4 g, 8.5 mmol) was added. The mixture was stirred for 24 hours at room temperature. Then ~ 50 g chipped ice was added and the reaction mixture was stirred until all the ice melted. The reaction mixture was extracted with chloroform (4 x 20 ml), and the combined extracts were dried over anhydrous $MgSO_4$. Concentration under reduced pressure followed by removal of excess pyridine under high vacuum yielded 3′-levulinoyl-5′-trityl-5-fluoro-2′-deoxyuridine as yellow oil. This product was re-dissolved in 80% acetic acid (20 ml) and heated for 20 min at 100 °C. The reaction mixture was then evaporated under reduced pressure, followed by repeated evaporation from benzene in order to remove traces of water and acetic acid. The resultant reaction mixture was then purified by flash chromatography on silica gel, using chloroform/methanol (40/1). 1.3 g of compound **(2)** was obtained as white powdery solid. Yield - 46%.

Stage 3. Preparation of 1-Stearoyl-2- 3″,3″-dimethyl-glutaroyl-sn-glycero-3-phosphocholine (3).

A solution of 1-stearoyl-sn-glycero-3-phosphocholine (1.8 g, 3.6 mmol), 3,3-dimethylglutaric anhydride (3.5 g, 25.2 mmol), sodium salt of 2-propylpentanoic

acid (0.5 g, 3.2 mmol) in N,N-dimethylformamide (20 ml) was heated for 5 hours at 90 °C. The reaction mixture was concentrated to dryness under high vacuum. The residue was dissolved in chloroform/methanol (2/1) 100 ml and washed with 0.1 N HCl (2 x 50 ml). After evaporation and flash chromatography on silica gel ($CHCl_3$/MeOH/H_2O/AcOH 60/30/4/1), 2 g of compound **(3)** was obtained as white crystalline solid. Yield - 87%.

Stage 4. Preparation of 1-Stearoyl-2-[5''-O-(-3'-O-levulinyl 5-fluoro-2'-deoxyuridine)-3'',3''-dimethylglutaroyl]-sn-glycero-3-phosphocholine (4).

To 1 g of compound **(3)** (1.5 mmol), 2.0M solution of oxalyl chloride in dichloromethane (20 ml, 4 mmol) was added. The mixture was stirred overnight at 20 °C. The acid chloride was separated from the oxalyl chloride by removal of the volatiles in vacuum followed by two cycles of dissolution in dry benzene (20 ml) and evaporation.

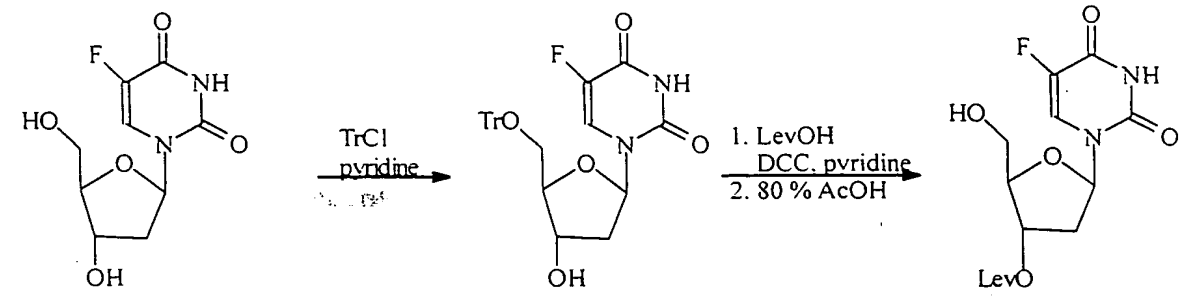
A solution consisting of compound **(2)** (700 mg, 2.15 mmol) and triethylamine (0.22 g, 2.17 mmol) in dichloromethane (10 ml) was added drop wise to solution of the acid chloride in dichloromethane (10 ml) at 0 °C. The mixture was left overnight at 20 °C, then the volatile solvants were removed and the residue was dissolved in chloroform/methanol (2/1, 50 ml) and washed with 0.1N HCl (20 ml). The solvent was removed and the residue was subjected to chromatography on silica gel using methanol/chloroform (10-33%) followed by water/methanol/chloroform (2/30/60) to yield 700 mg of compound **(4)**.

Yield - 48%.

Stage 5. Preparation of 1-Stearoyl-2-[(5''-O-5-fluoro-2'-deoxyuridine)-3'',3''–dimethylglutaroyl]-sn-glycero-3-phosphocholine (5).

Hydrazine monohydrate (0.25 ml, 4.8 mmol) in pyridine/acetic acid (3:2 v/v; 5 ml) was added to compound **(4)** (200 mg, 0.2 mmol) dissolved in pyridine (5 ml). After 2 min at 20 °C, the solution was cooled to 0 °C and 2-pentanone (2 ml) was added. The reaction mixture was then evaporated to dryness. Preparative TLC using water/methanol/chloroform (5/35/60) as the solvent yielded 120 mg of the final product **(5)**. Yield - 68%.

Scheme 2. Preparation of phospholipid derivatives of 2'-deoxy-5-fluorouridine (DP-5FUdR)



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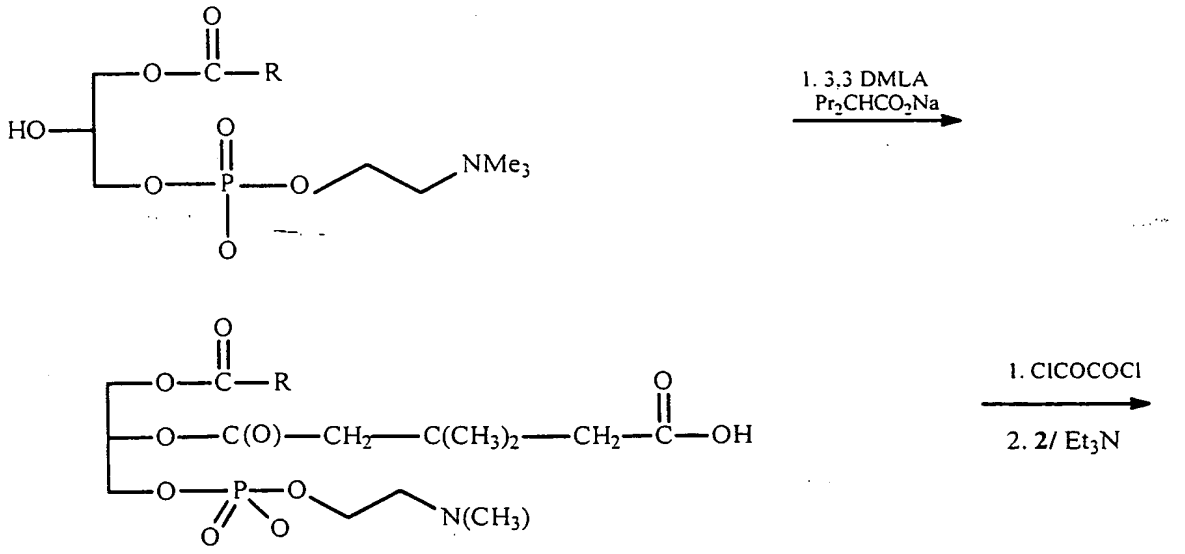

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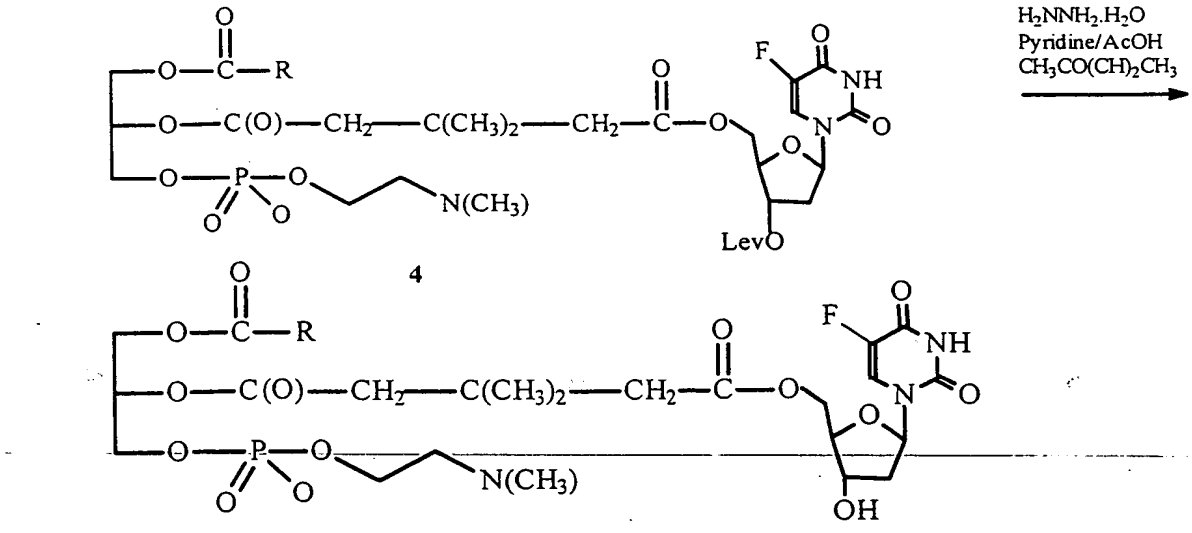


4 5

R- denotes a hydrocarbon chain having from 2 to 30 carbon atoms.

II. BIOLOGICAL EXAMPLES:

Example 4: Evaluation of cytotoxicity of methotrexate derivatives (in vitro studies)

The toxicity of methotrexate derivatives of the invention (DP-MTX) on cells in culture was evaluated. The doses required to inhibit the growth of cultured Friend cells (an erythroleukemic cell line) by 50%, over a 72 hour period (ID_{50}), were determined using a mitochondrial viability (MTT) endpoint assay.

Friend cells were seeded at a density of 10^4 cells/ml in RPMI medium supplemented with 10% FCS in 96 well plates. The cultured cells were incubated, during their linear growth phase, in the presence and absence of various concentrations of MTX derivatives. After 72 hours at 37^0C the cytotoxic effect on the cells was estimated by using the colorimetric MTT assay (Mosmann (1983) J. Immunol. Methods 65: 55-63) that measures mitochondrial reductase activity and serves for quantitative assessment of cellular viability.

Table 1

Compound	n	R	ID_{50}, nMol
MTX (control)	-	-	10
α-MTX			
DP-MTX-93	2	H	97.5
DP-MTX-129	3	H	97.5
DP-MTX-106	5	H	390
DP-MTX-142	7	H	3000
γ-MTX			
DP-MTX-71	2	$C_{12}H_{25}$	23

n is the number of carbon atoms in the linker between the MTX residue and the phospholipid moiety; **R** is the substituent at the γ- or α-carboxylic group of the α-MTX and γ-MTX compounds, respectively.

The following MTX derivatives were tested: 1-Stearoyl-2-[3-(α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine (denoted DP-MTX-93), 1-Stearoyl-2-[4-(α-MTX amido)-Butanoyl]-sn-Glycero-3- Phosphatidylcholine (denoted DP-MTX-129), 1-Stearoyl-2-[6-(α-MTX amido)-Hexanoyl]-sn-Glycero-3- Phosphatidylcholine (denoted DP-MTX-106), 1-Stearoyl-2-[8-(α-MTX amido)-Octanoyl]-sn-Glycero-3- Phosphatidylcholine (denoted DP-MTX-142), and 1-Stearoyl-2-[3-(α-dodecylate-γ-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine (denoted MTX-71).

DP-MTX-93, DP-MTX-129, DP-MTX-106 and DP-MTX-142 are α-MTX derivatives, namely molecules wherein MTX is linked to the lipid moiety through its α-carboxylic group. MTX-71, on the other hand, is a γ-MTX derivative, namely a molecule wherein MTX is linked through its γ-carboxyl instead of its α-carboxylic group. In addition, MTX-71 has an R residue, $C_{12}H_{25}$ group, linked to its α-carboxyl.

As can be seen in Table 1, MTX-71 is about 2-fold less active than the parent drug MTX, while being 4-fold more active compared to the α-MTX derivative, DP-MTX-93, which has the same bridging group.

Overall, as shown in Table 1, all tested DP-MTX derivatives, and especially DP-MTX-142, are less cytotoxic than the parent drug MTX (control). The tested alpha-DP-MTX derivatives were about 10- to 300-fold less toxic than MTX. These results supports the conclusion that alpha-DP-MTX derivatives are inactive prodrugs of the active species MTX.

Example 5: Anti-inflammatory activity of DP-MTX (in the carrageenan-induced rat paw oedema model)

The potential anti-inflammatory activities of various DP-MTX prodrugs were evaluated by employing the experimental model of carrageenan-induced paw oedema in the rat.

Carrageenan-induced rat paw edema is a widely employed animal model for acute inflammation. The objective of the study is to assess the potential

prophylactic effects of DP-MTX derivatives on the prevention of inflammatory swelling and, in particular, to compare the efficacy parameters with those obtained for MTX. The experimental set-up was as follows: Male Sprague-Dawley rats weighing 120-180g (supplied by Harlan Laboratories Breeding Center, Israel) were intraperitoneally (i.p.) injected with MTX or DP-MTX one hour prior to the induction of inflammation with carrageenan. MTX and the tested MTX-derivatives, DP-MTX-129 and DP-MTX-142, were applied at a dose level of 1 mg/kg body weight. Animals injected with the vehicle only served as a control group.

Paw edema was induced by a single sub-plantar injection of 0.1 ml 2% carrageenan in physiological saline, into the right hind paw of each test animal. Just prior to paw edema induction, the paw thickness of the test animals' right hind paws was measured in duplicate using a plethysmograph and micrometer to provide a baseline. At 3, 5 and 7 hours post carrageenan injection, the right paw thickness was measured in the same manner as before.

The assessment of potential anti-inflammatory activity was based on the relative reduction (%) in mean group values of carrageenan-induced right hind paw edema in animal groups treated with tested compounds versus respective values in the control group treated with vehicle only. The effect of DP-MTX on carrageenan-induced paw edema was evaluated in comparison to the effect of methotrexate by following severity of inflammation as determined by hind paw swelling.

Studies of this kind indicate that the phospholipid derivatives of methotrexate have higher therapeutic indices compared to methotrexate in the carrageenan-induced paw edema model for inflammation.

Example 6: *In-vivo* efficacy and toxicity studies of DP-MTX (in rat adjuvant-induced arthritis)

DP-MTX compounds were evaluated for efficacy and toxicological profile in the progression of adjuvant-induced arthritis (AA) in Lewis rats,

which is a widely employed animal model for the human rheumatoid arthritis (RA). The effects of DP-MTX were compared with those obtained with MTX.

Arthritis was induced in the left hind paws of male Lewis rats by sub-plantar injection of a suspension of *Mycobacterium butyricum* in paraffin oil. Test animals were intraperitoneally (i.p.) injected with either 0.02 mg/kg or 0.1 mg/kg of DP-MTX prodrug or the commercially available methotrexate (Abitrexate®). Dosing was initiated on the day of AA induction and thereupon continued as once-a-day repeated injections throughout a 3-week study period. An additional group of rats was treated with solvent only (1% propylene glycol and 0.01% Tween-80 in USP water) and served as a control group.

Assessment of the DP-MTX effects was based on relative changes in hind paw thickness, measured prior to the treatment, during and at termination of the study period. These effects were compared to the respective effects of MTX. In addition, determination of body weight gain at study termination was used as a measure of general systemic anti-inflammatory activity. Another parameter followed as an indication for the anti-inflammatory activity of the tested compounds, was the prevention of the characteristic changes in the hematologic pattern of AA in rats, such as leukocytosis, decreased MCV, lymphopenia, monocytosis, eosinopenia and thrombocytosis. In addition, all tested animals were inspected for clinical signs (e.g. histopathological patterns in the spleen, liver and intestines) to evaluate toxicity.

Results indicate that phospholipid derivatives of methotrexate are effective in suppression of AA-induced inflammatory phenotypes such as swelling, and evoke less pathological events.

Example 7: Anti-proliferative activity of DP-MTX (*in vitro* study)

The anti-proliferative activities of various DP-MTX prodrugs were tested in vitro on cultured gliosarcoma cells as follows. Rat gliosarcoma 9L/LacZ cells are grown to confluence in DMEM medium supplemented with 10% FCS. Confluent cultured cells are detached using brief enzymatic digestion with 0.1% trypsin and plated onto 96- or 24-well tissue culture plates

at a concentration of 10^5 cells/ml. 42 hours later, the plated cells reach their exponential growth rate. MTX or MTX-derivatives are added at concentrations ranging from 1 to 1000 nM. As a control serve cells exposed to vehicle only. The plates are incubated for different time periods up to 96 hours. At specified time-points the amount of lactate dehydrogenase (LDH) in the growth condition medium is determined in order to assess toxicity. In addition, the metabolic activity of the cells is assayed by using the colorimetric MTT assay (Mosmann (1983) J. Immunol Methods 65: 55-63) as a measurement for cell viability.

The gliosarcoma cell population doubling time is also analyzed. Cells are seeded at a concentration of 1.5 X 10^4 cells/cm^2 into 24 well tissue culture plates in triplicates. At intervals of 6-24 hours, Cells detached after gentle trypsinization are exposed to Trypan blue (0.1%) and viable cells (not stained by Trypan blue) are counted in a hemocytometer. The population doubling time (PDT) in the exponential growth phase is determined by the following equation: $PDT = (t - t_0)\log 2/(\log N - \log N_0)$

Where t_0 is the time at the first counting; t is the time at a second counting; N is the number of viable cells at time t; and N_0 is the number of viable cells at time t_0.

Results of these measurements indicate that MTX derivatives are effective in inhibiting the gliosarcoma cell growth and/or survival, thus, demonstrating that the tested DP-MTX compounds are useful as anti-proliferative agents.

Example 8: *In vivo* efficacy studies of DP-MTX (in the experimental allergic encephalomyelitis (EAE) model system)

The effects of DP-MTX compounds on sites of inflammation within the central nervous system are assessed in an animal model. The chosen model system is experimental allergic encephalomyelitis (EAE) which is a known animal model for multiple sclerosis (MS).

In a preliminary set of experiments EAE was induced in SJL/J mice by either one of two schemes: a) active induction by injecting mice via the footpads with proteolipid protein (PLP $_{139-151}$) emulsified in complete Freund's adjuvant (CFA) as described by Weinberg et al. (J. Immunol. (1999) 162: 1818-1826), or b) passive induction, namely adoptive transfer of EAE accomplished by transferring reactive cells from a donor. Briefly, PLP-reactive lymph node cells are drawn from mice inoculated with PLP-CFA, 10 days post inoculation. The cells are incubated in vitro for 4 days with soluble PLP, then collected, washed and transferred to naive mice.

The tested DP-MTX compounds are injected either i.p or i.v. at 8, 10 or 12 days post inoculation in the case of the active induction of EAE, or at days 2, 4 and 6 after cell transfer in the passive induction protocol. According to both procedures, the animals are sacrificed 4 hours after injection of the tested DP-MTX compound. Blood samples are collected and the animals' brains and spinal cords are excised and homogenized. The levels of MTX in serum and tissue extracts of the treated animals are determined. Briefly, samples of brain or spinal cord are suspended in three volumes of 5 mg/ml sodium ascorbate. Samples are homogenized, sonicated and centrifuged at 27000g for 30 min. Supernatants are placed in boiling water bath for 10 min. and then centrifuged at 3000g for 1 min. The obtained clear supernatants are used for determination of free MTX. Abbot's TDX method and kit are used to determine the levels of MTX in serum and tissue extracts.

A group of mice with EAE are kept for a longer time while receiving leucovorin daily and is watched for EAE symptoms.

EAE severity is evaluated statistically by following two main parameters: a) neurological symptoms scored according to a standard score in which: 0- no clinical signs; 1- limp tail; 2- ataxia; 3- hind limb paralysis; 4- complete paralysis and incontinence; 5- moribund and death; and

b) histopathological symptoms which are evaluated by using sections of the brain stained with hematoxylin-eosin for detection of mononuclear cell infiltration and with luxol-fast for the detection of demyelination.

Results have shown that the tested DP-MTX compounds reach the site of inflammation and exert their therapeutic effect in inhibiting the clinical signs of EAE.

Conclusion: DP-MTX may be useful in treatment of CNS autoimmune diseases and inflammation.

Example 9: Effects of DP-MTX and DP-5FUdR compounds in restenosis model system in rats.

The anti-proliferative effects of compounds of the invention are assessed in a model system for restenosis in rats. Restenosis is a phenomenon of neointimal formation occurring following percutaneous transluminal coronary angioplasty (PTCA) procedures.

Experimental neointimal formation is induced by balloon injury in rats according to the following procedure. Male Spraque-Dawley rats (380-450 g) are anesthetized by inhalation of halothane and dinitric oxyde. After the right common carotid artery and the right external carotid artery are exposed, a 2F Fogarty arterial embolectomy catheter (Baxter Healthcare, Santa Ana, CA) is inserted into the lumen of the right external carotid artery and is guided to a fixed distance (about 5 cm). The balloon is inflated with saline and is withdrawn at a constant rate back to a point proximal to the site of insertion. This procedure is repeated three times.

The effect of the balloon injury is assessed 2 to 4 weeks following the above-described treatment. The left (control, untreated) and right (experiment, treated) carotid arteries of rats under pental overdose, are perfused in situ with 4% formaline, pH 7.4 and then excised. Each of the left and right arteries is cut at three segments: upper, middle and lower, and then fixed in formaline. Cross-sections are stained with van Gieson's stain and the intima and media thickness is evaluated under a microscope. A higher calculated ratio of intima/media in the treated (right) artery in comparison to the control (left) artery, is an indication of neointimal formation and development of restenosis.

Various DP-MTX and DP-5-FUdR compounds are tested for preventive and/or curative effects on restenosis by carrying-out the following schemes:

a) Preventive procedure – the tested compound is daily administered starting from 7 to 3 days prior to the induced balloon injury, and up to the day of the induced injury.

b) Curative procedure – the tested compound is daily administered starting from the day of induced balloon injury and up to 14 to 30 days after the induced injury.

Peroral- and parenteral- , such as subcutaneous, modes of administration may be employed in both the preventive and curative procedures.

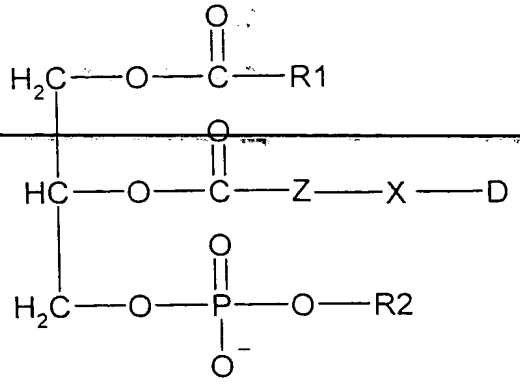
In yet another regimen, a combination of the preventive and the curative procedures is tested in the rat model system for restenosis.

Conclusion: Both DP-MTX and DP-5-FUdR compounds are promising agents for inhibition of intimal proliferation and may be useful in clinical procedures.

While the present invention has been particularly described, persons skilled in the art will appreciate that many variations and modifications can be made. Therefore, the invention is not to be construed as restricted to the particularly described embodiments, rather the scope, spirit and concept of the invention will be more readily understood by reference to the claims which follow.

CLAIMS:

1. A prodrug of the general formula I



Formula I

or a pharmaceutically acceptable salt thereof, wherein:

R1 is a saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms;

R2 is H or a phospholipid head group;

Z is saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 15 carbon atoms, which may include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms;

X is a covalent bond or selected from the group consisting of O, S, NH and C(O) groups; and

D is a residue of an anti-proliferative drug,

wherein the bound anti-proliferative drug residue is an inactive form of the drug which is selectively activated in cells and tissues with elevated phospholipase activity.

2. The prodrug according to claim 1, wherein the anti-proliferative drug is methotrexate or pharmaceutically acceptable derivatives thereof.

3. The prodrug according to claim 2, wherein the methotrexate or pharmaceutically acceptable derivative thereof is attached to X through the α carboxylic group of methotrexate.

4. The prodrug according to claim 3, wherein said pharmaceutically acceptable derivatives of methotrexate are derivatives wherein the hydrogen atom of the γ carboxylic group of methotrexate is substituted for CH_3 or $C_{12}H_{25}$.

5. The prodrug according to claim 2, wherein the methotrexate or pharmaceutically acceptable derivative thereof is attached to X through the γ carboxylic group of methotrexate, and wherein the hydrogen atom of the α carboxylic group of methotrexate is substituted by a saturated or unsaturated, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms.

6. The prodrug according to claim 1, wherein the anti-proliferative drug is 2'-deoxy-5-fluorouridine and pharmaceutically acceptable derivatives thereof.

7. The prodrug according to claim 1, wherein an ester bond at position sn-2 of the phospholipid of the general formula I is cleaveable by a phospholipase.

8. The prodrug according to claim 7, wherein said phospholipase is phospholipase A_2 (PLA_2).

9. The prodrug according to claim 1, wherein R1 is an hydrocarbon chain having from 5 to 20 carbon atoms.

10. The prodrug according to claim 1, wherein R1 is an hydrocarbon chain having 15 or 17 carbon atoms.

11. The prodrug according to claim 1, wherein R2 is selected from the group consisting of choline, ethanolamine, inositol and serine.

12. The prodrug according to claim 1 selected from the group consisting of:

1-Stearoyl-2-[3-(α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[3-(γ-dodecylate-α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[4-(α-MTX amido)-Butanoyl]-sn-Glycero-3-Phosphatidylcholine,

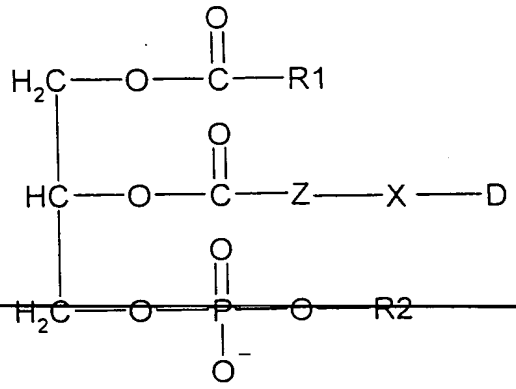
1-Stearoyl-2-[6-(α-MTX amido)-Hexanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[8-(α-MTX amido)-Octanoyl]-sn-Glycero-3-Phosphatidylcholine;

1-Stearoyl-2-[8-(γ-dodecylate-α-MTX amido)-Octanoyl]-sn-Glycero-3-Phosphatidylcholine, and

1-Stearoyl-2-[(5''-2'-deoxyuridine)-3'',3''–dimethyl]-glutaroyl-sn-Glycero-3-Phosphatidylcholine.

13. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a prodrug of the general formula I



Formula I

or a pharmaceutically acceptable salt thereof, wherein:

R1 is a saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms;

R2 is H or a phospholipid head group;

Z is saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 15 carbon atoms, which may include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms;

X is a covalent bond or selected from the group consisting of O, S, NH and C(O) groups; and

D is a residue of an anti-proliferative drug,

wherein the bound anti-proliferative drug residue is an inactive form of the drug which is selectively activated in cells and tissues with elevated phospholipase activity.

14. The pharmaceutical composition according to claim 13, wherein the anti-proliferative drug is methotrexate or pharmaceutically acceptable derivatives thereof.

15. The pharmaceutical composition according to claim 14, wherein the methotrexate or pharmaceutically acceptable derivative thereof is attached to X through the α carboxylic group of methotrexate.

16. The pharmaceutical composition according to claim 15, wherein said pharmaceutically acceptable derivatives of methotrexate are derivatives wherein the hydrogen atom of the γ carboxylic group of methotrexate is substituted for CH_3 or $C_{12}H_{25}$.

17. The pharmaceutical composition according to claim 14, wherein the methotrexate or pharmaceutically acceptable derivative thereof is attached to X through the γ carboxylic group of methotrexate, and wherein the hydrogen atom of the α carboxylic group of methotrexate is substituted by a saturated or unsaturated, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms.

18. The pharmaceutical composition according to claim 13, wherein the anti-proliferative drug is 2'-deoxy-5-fluorouridine and pharmaceutically acceptable derivatives thereof.

19. The pharmaceutical composition according to claim 13, wherein an ester bond at position sn-2 of the phospholipid of the general formula I is cleaveable by a phospholipase.

20. The pharmaceutical composition according to claim 19, wherein said phospholipase is phospholipase A_2 (PLA_2).

21. The pharmaceutical composition according to claim 13, wherein R1 is an hydrocarbon chain having from 5 to 20 carbon atoms.

22. The pharmaceutical composition according to claim 13, wherein R1 is an hydrocarbon chain having 15 or 17 carbon atoms.

23. The pharmaceutical composition according to claim 13, wherein R2 is selected from the group consisting of choline, ethanolamine, inositol and serine.

24. The pharmaceutical composition according to claim 13, wherein said compound of the general formula I is selected from the group consisting of:

1-Stearoyl-2-[3-(α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[3-(γ-dodecylate-α-MTX amido)-Propanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[4-(α-MTX amido)-Butanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[6-(α-MTX amido)-Hexanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[8-(α-MTX amido)-Octanoyl]-sn-Glycero-3-Phosphatidylcholine,

1-Stearoyl-2-[8-(γ-dodecylate-α-MTX amido)-Octanoyl]-sn-Glycero-3-Phosphatidylcholine, and

1-Stearoyl-2-[(5''-2'-deoxyuridine)-3'',3''–dimethyl]-glutaroyl-sn-Glycero-3-Phosphatidylcholine.

25. The pharmaceutical composition according to any one of claims 13 to 24 further comprising an additional anti-neoplastic agent.

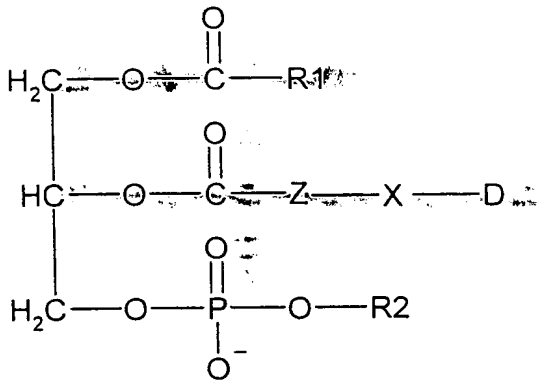
26. The pharmaceutical composition according to any one of claims 13 to 25, in the form of solutions, suspensions, capsules, tablets, aerosols, gels, ointments or suppositories.

27. The pharmaceutical composition according to any one of claims 13 to 25 for oral, ocular, nasal, parenteral, topical or rectal administration.

28. The pharmaceutical composition according to claim 27 for intravenous administration.

29. The pharmaceutical composition according to claim 27 for oral administration.

30. Use for the preparation of a pharmaceutical composition of a prodrug of the general formula I



Formula I

or a pharmaceutically acceptable salt thereof, wherein:
R1 is a saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 30 carbon atoms;
R2 is H or a phospholipid head group;
Z is saturated or unsaturated, straight-chain or branched, substituted or unsubstituted hydrocarbon chain having from 2 to 15 carbon atoms, which may

include cyclic elements, and optionally is interrupted by one or more atoms selected from oxygen and sulfur atoms;

X is a covalent bond or selected from the group consisting of O, S, NH and C(O) groups; and

D is a residue of an anti-proliferative drug,

wherein the bound anti-proliferative drug residue is an inactive form of the drug which is selectively activated in cells and tissues with elevated phospholipase activity.

31. A method for treatment of a disease or disorder related to an inflammatory condition comprising administering to a patient in need thereof a therapeutically effective amount of a pharmaceutical composition according to any one of claims 13 to 29.

32. The method according to claim 31, wherein said disease or disorder related to an inflammatory condition is selected from the group consisting of granulomatous disease, arthritis, rheumatoid arthritis, systemic sclerosis, asthma, psoriasis, systemic lupus erythematosus, inflammatory bowel syndrome and migraines.

33. A method for treatment of a disease or disorder related to a degenerative or atrophic condition comprising administering to a patient in need thereof a therapeutically effective amount of a pharmaceutical composition according to any one of claims 13 to 29.

34. The method according to claim 33, wherein said disease or disorder related to a degenerative or atrophic condition is a central or peripheral neurological disease or disorder.

35. The method according to claim 33, wherein said disease or disorder related to a degenerative or atrophic condition is selected from the

group consisting of autoimmune, cerebrovascular and neurodegenerative diseases and disorders such as idiopathic dementia, vascular dementia, multi-infarct dementia, traumatic dementia, Alzheimer's disease, Pick's disease, Huntington's disease, dementia paralitica, Parkinson's disease, diabetic neuropathy, multiple sclerosis, amyotrophic lateral sclerosis, acute ischemia of the optic nerve, age-related macular degeneration, stroke and trauma.

36. A method for treatment of a disease or disorder related to uncontrolled cell growth comprising administering to a patient in need thereof a therapeutically effective amount of a pharmaceutical composition according to any one of claims 13 to 29.

37. The method according to claim 36, wherein said disease or disorder related to uncontrolled cell growth is a neoplastic growth.

38. The method according to claim 37 wherein said neoplastic growth is a primary or a secondary tumor.

39. The method according to claim 36, wherein said disease or disorder related to uncontrolled cell growth is selected from the group consisting of psoriasis, lymphocytic leukemia, acute myelogenous leukemia, meningeal leukemia, non-Hodgkin's lymphomas, mycosis fungoides, osteosarcoma, hydatidiform mole, trophoblastic diseases such as chorioadenoma destruens and choriocarcinoma, epidermoid cancers of the head and neck, and carcinomas of the breast, liver, lung, colon, ovary, cervix, urinary, bladder, prostate, pancreas, the gastrointestinal tract and the oropharyngeal areas.

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